Measurement of Vasomotor Reserve in the Transcranial Doppler-CO\textsubscript{2} Test Using an Ultrasound Contrast Agent (Levovist)

Matthias Rohrberg, MD; Rudolf Brodhun, MD

Background and Purpose—Determination of vasomotor reserve (VMR) with the transcranial Doppler-CO\textsubscript{2} (TCD-CO\textsubscript{2}) test is used to assess the risk of impending cerebral ischemia in patients with high-grade stenosis or occlusion of the internal carotid artery. In patients with a poor temporal window, however, this examination is limited. The aim of this study therefore was to examine whether the use of an ultrasound contrast agent (USCA) influences the results of the TCD-CO\textsubscript{2} test.

Methods—In the first part of the study, 6 control subjects and 20 patients were examined with the TCD-CO\textsubscript{2} test. The VMR was determined first without the application of a contrast agent and then with continuous infusion of an USCA (Levovist, 300 mg/mL, 1 mL/min). In the second part of the study, 2 tests without USCA were performed in each of 13 patients and 2 tests with USCA infusion were performed in each of 12 patients. Statistical analysis included differences between the VMR determined with the 2 comparative measurements (ΔVMR), the mean (MΔVMR), and SD.

Results—Based on the mean difference, the TCD-CO\textsubscript{2} test produced the same results with and without USCA (MΔVMR 1.8%), although the differences showed a wide distribution (2 SDs, ±20.7%). Similar spreads were seen in repeated determinations of VMR in the same patient without USCA (2 SDs, ±20.0%), whereas the distribution under continuous USCA infusion was considerably smaller (2 SDs, ±8.2%).

Conclusions—The TCD-CO\textsubscript{2} test can be performed with continuous infusion of an USCA without influencing the results. Even with a good temporal window, the results of the TCD-CO\textsubscript{2} test show better reproducibility and thus better reliability if an USCA is used. (Stroke. 2001;32:1298-1303.)

Key Words: carotid artery disease ■ cerebral blood flow ■ cerebral ischemia ■ contrast media ■ ultrasonography, Doppler, transcranial

Determination of the vasomotor reserve (VMR) is a useful method for risk assessment of cerebral ischemia in patients with high-grade stenosis or occlusion of the internal carotid artery (ICA). Patients with steno-occlusive disease in whom cerebrovascular reactivity is impaired are at high risk to suffer cerebral ischemia.\textsuperscript{1,2} The VMR is based on the dilatative capacity of the intracerebral arterioles.\textsuperscript{3} The residual dilative capacity of the cerebral arterioles can be measured quantitatively with the transcranial Doppler-CO\textsubscript{2} (TCD-CO\textsubscript{2}) test. Arterial hypercapnia leads to dilatation of the arterioles with a resultant increase in the mean flow rate ($V_{\text{mean}}$) in the basal intracranial arteries, eg, in the middle cerebral artery (MCA). The alteration in the cerebral blood flow in response to the CO\textsubscript{2} stimulus is dependent on the cerebral perfusion pressure\textsuperscript{4} (Figure 1). With decreasing perfusion pressure, eg, in the case of a preceding obstruction in the arteries supplying the brain, there is an increasing leftward shift in the S-shaped curve, ie, toward smaller changes in cerebral blood flow with increasing P\textsubscript{CO\textsubscript{2}}. If, in hypercapnia, an increase in the P\textsubscript{CO\textsubscript{2}} by 1% by volume leads to a flow increase of <10% in the MCA, the VMR is considered to be diminished. If, in hypocapnia, the flow also does not decrease by >10% when there is a decrease in the P\textsubscript{CO\textsubscript{2}} by 1% by volume, the VMR is by definition “exhausted” (the so-called 2-sided method).\textsuperscript{3}

Because the TCD-CO\textsubscript{2} test requires transtemporal examination of the MCA, an inadequate ultrasonic window is a common problem, particularly in elderly female patients. Even in younger patients, <50 years old, an inadequate temporal window can be expected in 5% to 10%; in women >75 years old, the proportion can be as high as 50%.\textsuperscript{3} Reliable determination of $V_{\text{mean}}$ is not possible given a weak signal in the MCA, and assessment of the VMR therefore is either not possible or very unreliable. Intravenous injection of an ultrasound contrast agent (USCA) that is not eliminated via the lungs (eg, Levovist) considerably improves the quality of the Doppler signal even if penetration of the temporal bone is poor.\textsuperscript{5–10} Therefore, it seemed to be logical to perform the
TCD-CO\textsubscript{2} test with such an agent. The effect of a single-bolus injection is only of short duration. Immediately after the injection, the rapid increase in the concentration leads to excessive enhancement of the ultrasound signal (so-called blooming effect), which then gradually weakens with declining concentrations.\textsuperscript{11} Evaluation of the TCD-CO\textsubscript{2} test requires determination of the flow signal in the MCA for 10 to 15 minutes and thus a constant concentration and effect of the USCA over this period of time. This can be achieved through a continuous injection with an infusion pump.\textsuperscript{6,12}

However, the question of whether the use of echo-enhancing contrast agents leads to an increase in the velocity determined by Doppler ultrasound is controversial in the literature. Although Melany et al\textsuperscript{13} came to the conclusion that USCAs do not significantly influence the measured velocity, Forsberg et al\textsuperscript{14} and Fürst et al\textsuperscript{15} described an increase of 20\% to 45\% in the maximum Doppler shift. These possible differences of the measured $V_{\text{mean}}$ could influence the results of the TCD-CO\textsubscript{2} test. However, there are, to our knowledge, no data available that address this question, and the validity of the TCD-CO\textsubscript{2} test with an USCA remains to be established. In the present study, we therefore compared calculation of the VMR with and without the use of an USCA.

**Subjects and Methods**

The MCA was insonated transtemporally by means of 2-MHz pulse-wave Doppler probes affixed to the patient’s head, and the $V_{\text{mean}}$ was continuously recorded. Pure carbogen (5\% CO\textsubscript{2} and 95\% O\textsubscript{2}) was delivered via face mask; this produced the required increase in the arterial P CO\textsubscript{2} of 1\% by volume. The change in the $V_{\text{mean}}$ in the MCA in hypercapnia was measured, and the VMR was calculated as the relative percent increase in $V_{\text{mean}}$ (Figure 2). For further evaluation of pathological results (increase in $V_{\text{mean}} < 10\%$), in accordance with the “2-sided” method, the flow change in the MCA in hypocapnia (hyperventilation) was also measured. To observe a uniform examination protocol for all measurements, these results were not, however, included in the study.

The TCD was performed with a Neuroguard Doppler system with integrated software for the CO\textsubscript{2} test and the possibility of simultaneous monitoring of both MCAs. Two Doppler probes were fastened to the patient’s head over the temporal window using a special fixation device that permitted stable fixation of the probes. If possible, both MCAs were examined simultaneously at a depth of between 50 and 60 mm, and the intensity-weighted $V_{\text{mean}}$ was recorded continuously. Because a stable Doppler signal without the use of the contrast medium was necessary for comparison of the results with and without an USCA, subjects with a bilateral poor temporal window were withdrawn from the study and subjects with a good temporal window only on 1 side were examined unilaterally. When the $V_{\text{mean}}$ was stable for $\geq 5$ minutes, the patients were given pure carbogen via a mask with a valve and reservoir bag. After stabilization of the new $V_{\text{mean}}$ for a few minutes (Figure 2), the test was terminated. If there was little or no change in $V_{\text{mean}}$ in hypercapnia, the test was not terminated before 10 minutes had elapsed. The integrated software was used to determine the $V_{\text{mean}}$ in normocapnia ($V_{\text{Normo}}$) and the $V_{\text{mean}}$ in hypercapnia ($V_{\text{Hyper}}$), and the VMR was calculated with the formula shown in Figure 2. The test
was repeated in the same patient or subject after an interval of 15 minutes during which the flow in the MCA had returned to normal again. The position of the Doppler probes was not changed between the tests.

Levovist was infused with an IVAC Medical Systems P 4000 infusion pump specially configured for the administration of high flow rates and with Medrad connecting tubes. For each test, 4 g Levovist (Schering) was injected into a cubital vein in a concentration of 300 mg/mL with a flow rate of 1 mL/min (60 mL/h) via an indwelling cannula. If it was necessary to prolong the examination time, Levovist was administered again continuously in a concentration of 300 mg/mL up to the end of the examination.

The examinations were performed between July 1 and December 31, 1999, in the Department of Neurology and Neuropsychiatry of the Asklepios Kliniken Schildautal, Seesen, Germany.

Part 1
In the first part of the study, the results of the TCD-CO2 test with and without Levovist were compared in the same subject or patient.

For this portion of the study, we examined 6 healthy volunteers and 20 patients with carotid stenosis or occlusion in whom performance of the TCD-CO2 test was indicated (Table 1). The patients gave informed consent to participate in the study. Inclusion criteria were high-grade stenosis (>70%) or occlusion of the extracranial or intracranial ICA, the common carotid artery, or the brachiocephalic trunk as confirmed with Doppler and duplex ultrasound studies as well as a good temporal ultrasonic window, so that a stable envelope of the MCA when the examination was performed without contrast enhancement. The examination was not carried out in the first 6 weeks after acute cerebral ischemia. The exclusion criteria were obstructive lung disease, angina pectoris, myocardial infarction in the past 6 months, intracerebral hemorrhage in the past 3 months, and cerebral infarction in the past 6 weeks. Lack of cooperation or failure to give informed consent were further exclusion criteria.

In 1 patient and 1 control subject, the MCA was examined only unilaterally, so that a total of 50 comparative measurements were performed.

Part 2
The second part of the study was further divided into 2 sections. The inclusion and exclusion criteria were the same as for the first part. In

### TABLE 1. Participants in Part 1: Comparison of Repeated Measurements With and Without Levovist

<table>
<thead>
<tr>
<th>Participants, n</th>
<th>Sex, n</th>
<th>Age, y</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, 6</td>
<td>M, 4</td>
<td>24–31</td>
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<tr>
<td>F, 2</td>
<td>Mean 28.0</td>
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<td></td>
</tr>
<tr>
<td>Patients, 20</td>
<td>M, 14</td>
<td>53–72</td>
<td>Unilateral ICA occlusion (n=13)</td>
</tr>
<tr>
<td>F, 6</td>
<td>Mean 64.0</td>
<td></td>
<td>Unilateral ICA stenosis* (n=4)</td>
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<td></td>
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<td>Bilateral ICA stenosis* (n=3)</td>
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</tbody>
</table>

*Stenosis of >70%.

### TABLE 2. Patients in Part 2: Repeatability of the TCD-CO2 Test With and Without Levovist

<table>
<thead>
<tr>
<th>Section</th>
<th>Sex, n</th>
<th>Age, y</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Repeated Measurements</td>
<td>M, 9</td>
<td>39–80</td>
<td>Unilateral ICA occlusion (n=4)</td>
</tr>
<tr>
<td>Without Levovist, 13</td>
<td>F, 4</td>
<td>Mean 64.8</td>
<td>Bilateral ICA occlusion (n=1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unilateral ICA stenosis* (n=6)</td>
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<tr>
<td></td>
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<td></td>
<td>Bilateral ICA stenosis* (n=1)</td>
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<td></td>
<td></td>
<td></td>
<td>Trunk stenosis* (n=1)</td>
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<tr>
<td>B: Repeated Measurements</td>
<td>M, 10</td>
<td>42–75</td>
<td>Unilateral ICA occlusion (n=9)</td>
</tr>
<tr>
<td>With Levovist, 12</td>
<td>F, 2</td>
<td>Mean 61.1</td>
<td>Unilateral ICA stenosis* (n=1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bilateral ICA stenosis* (n=2)</td>
</tr>
</tbody>
</table>

*Stenosis of >70%.

### TABLE 3. Differences in the VMR Among Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Comparison With and Without Levovist (n=50), %</th>
<th>Repeatability Without Levovist (n=22), %</th>
<th>Repeatability With Levovist (n=21), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>M&lt;sub&gt;VMR&lt;/sub&gt;</td>
<td>1.8</td>
<td>4.8</td>
<td>2.2</td>
</tr>
<tr>
<td>P&lt;sub&gt;0.228&lt;/sub&gt;*</td>
<td>P&lt;sub&gt;0.034&lt;/sub&gt;*</td>
<td>P&lt;sub&gt;0.025&lt;/sub&gt;*</td>
<td></td>
</tr>
<tr>
<td>2 SD&lt;sub&gt;VMR&lt;/sub&gt;</td>
<td>±20.7</td>
<td>±20.0</td>
<td>±8.2</td>
</tr>
<tr>
<td>P&lt;sub&gt;0.0002†&lt;/sub&gt;</td>
<td></td>
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</table>

* t test for paired observations. †F test.

section A, we compared the repeated measures of VMR in 13 patients in whom the test was performed twice without Levovist (Table 2). The MCA was examined only unilaterally in 4 patients, so that 22 comparative measurements were performed.

In section B, twin measurements with infusion of Levovist were carried out in 12 patients. In 3 patients, the MCA was examined only unilaterally, so that a total of 21 comparative measurements were obtained (Table 3).

### Statistical Methods
Comparison of the 2 test results in the respective parts of the study was performed according to the method of Bland and Altman. In this method, the difference between the 2 test results (ΔVMR=VMR<sub>1</sub>−VMR<sub>2</sub>) is plotted against their mean (M<sub>VMR</sub>) and the mean difference (M<sub>ΔVMR</sub>), and the interval of 2 SDs from the mean is calculated as a measure of the agreement between the results (M<sub>ΔVMR</sub>±2 SD<sub>ΔVMR</sub>). Because evaluation of the data using the Kolmogorov-Smirnov test showed a normal distribution of the data (P>0.66), the VMR of the repeated measurements for each study group was tested by using the t test for paired observations. In the second part of the study, the F test was performed to compare the variance of ΔVMR in sections A and B.

### Results
The results of the individual subgroups are summarized in Table 3 and presented graphically in Figures 3 to 5. In the first part, we compared the results of the TCD-CO2 test obtained in the same patient or subject with and without the infusion of Levovist. The mean difference between the VMR values obtained with the 2 procedures was 1.8% (ie, when the measurement was performed without Levovist, the VMR was on average 1.8% higher, but this difference was not significant in the t test; P=0.228). In general, it can be assumed that 95% of the differences in the VMR are within the range of ±20.7% of this mean (2 SDs).
In the second part of the study, the agreement was examined between repeated measurements (repeatability) with the TCD-CO₂ test in the same patient. In section A, the mean difference in the VMR on repeated measurements without Levovist was 4.8% (\(P=0.034\), \(t\) test), indicating that the second measurement tended to be of a higher value. The distribution of the differences was ±20.0% (2 SDs) and therefore was within the same range as in the first part. After the infusion of Levovist in section B, the mean difference between the repeated measurements was 2.2% (\(P=0.025\), \(t\) test), and the distribution was ±8.2% (2 SDs). The \(t\) test for paired observations showed in both sections a remarkable difference for repeated measurements with a tendency for higher second measurements. However, the level of significance was low (\(P=0.034\) and \(P=0.025\)). The differences in the variation of \(\Delta\)VMR in the 2 sections were highly significant as demonstrated by the \(F\) test (\(P=0.0002\)).

**Discussion**

Various procedures are used to determine the VMR. A reliable method of determining the cerebrovascular reserve capacity and the least invasive method for the patient are Doppler ultrasound procedures such as the TCD-CO₂ or acetazolamide test. In contrast to the TCD-CO₂ test, in the acetazolamide test there may be nonresponders or compensatory hyperventilation by the patient. Widder³ therefore recommends performance of the TCD-CO₂ test for clarification of pathological findings.

We were able to show that in patients with a sufficient temporal bone window, the TCD-CO₂ test can be performed under a continuous infusion of Levovist. In the statistical mean, there was no significant difference between VMR measurements with and without USCA, and the comparative measurements showed a good mean agreement. However, there were occasional considerable individual differences. The variation in \(\Delta\)VMR (2 SDs) was ±20.7%. There are 2 possible explanations for this: on the one hand, the differences might be due to the USCA, but on the other hand, these differences may reflect errors caused by poor repeatability (reliability) inherent in the TCD-CO₂ test.

In a second part of the study, we therefore examined the repeatability of the TCD-CO₂ test. Here we found that on repeated measurements in the same patient and probe position without the use of an USCA, the differences showed a
similarly wide distribution (2 SDs, ±20.0%). We thus were able to show that the TCD-CO$_2$ test without an USCA has a relatively poor reproducibility, thus explaining the wide spread of the differences in the comparison between the tests with and without the contrast medium. However, variation fell significantly after an infusion of Levovist (2 SDs, ±8.2%), thus indicating a better reproducibility of the test results. In our opinion, even with an apparently good temporal window, the use of an USCA produces a more stable Doppler signal and improves the reproducibility of the measurements.

In the second part of our study, there was a slight but significant difference in the repeated measurements, indicating a systematic error. This may in part be explained by the short interval of 15 minutes between the 2 measurements. If the second TCD-CO$_2$ test is repeated too quickly, the flow of the MCA may in some cases not have returned to normal. We therefore recommend choosing an interval of ≥15 minutes before repeating the TCD-CO$_2$ test twice in the same patient.

Our results show that the use of an USCA in the TCD-CO$_2$ test does not produce substantial systematic differences compared with examination without an echo-enhancing contrast agent. Because the performance of the test without the contrast medium does not provide reliable results in subjects with poor penetration of the temporal bone window, a corresponding comparison of the tests is not possible in such subjects. Nevertheless, it can be assumed that in patients with an inadequate temporal window, the VMR can be determined more reliably using a contrast agent. Alternative and more invasive methods, such as xenon-enhanced computed tomography or positron emission tomography, are not necessary.

However, the results also show that even with an apparently good temporal window, the repeatability, and thus the diagnostic reliability, of the TCD-CO$_2$ test is significantly improved when the test is performed with the continuous administration of an USCA. We therefore recommend, particularly in cases of pathological or borderline findings, repeating the test with an USCA to be able to make an unambiguous and reliable diagnostic statement.

The TCD-CO$_2$ test provides important information about patients with steno-occlusive disease of the cerebral arteries, although the studies to date that have investigated impaired VMR as an indication for cerebrovascular surgery have not been able to provide definitive results as to the indication for surgery. After the EC-IC Bypass Study in 1985, the use of extracranial-intracranial bypass surgery for occlusion of the ICA has been highly controversial. A number of studies have shown that patients with an exhausted VMR are at an increased risk of cerebral ischemia. After extracranial-intracranial bypass surgery, the previously impaired cerebrovascular and oxygen metabolic reserve is normalized. Thus, determination of the VMR may help in identifying those patients most likely to profit from extracranial-intracranial bypass surgery. In patients with asymptomatic stenosis of the ICA, Gur et al were able to show that an impaired VMR is associated with a high risk of cerebral ischemia. Nevertheless, the use of VMR in the selection of patients for carotid endarterectomy needs to be studied.

The results of our study show that the TCD-CO$_2$ test can be improved by the use of an USCA. This provides a more objective test method for risk assessment in patients with steno-occlusive disease of the ICA, especially in patients with poor penetration of the temporal bone window.

References
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*Stroke*. 2001;32:1298-1303
doi: 10.1161/01.STR.32.6.1298

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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